16S and 18S rRNA gene amplicon sequence links and metadata from microbial mesocosms spiked with 2-heptyl-4-quinolone (HHQ) experiments, Bergen, Norway, May 2017

Website: https://www.bco-dmo.org/dataset/753232
Data Type: experimental
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Version Date: 2019-01-21

Project
» Collaborative Research: Building a framework for the role of bacterial-derived chemical signals in mediating phytoplankton population dynamics (HHQSignals)

Contributors	Affiliation	Role
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Coverage

Spatial Extent: **Lat**:60.221 **Lon**:5.281
Temporal Extent: 2017-05-16 - 2017-05-30

Dataset Description

This dataset includes NCBI links and metadata for 16S and 18S rRNA gene amplicon sequences from microbial mesocosms spiked with 2-heptyl-4-quinolone (HHQ) experiments, Bergen, Norway, May 2017.

Methods & Sampling

Mesocosm setup and sampling:

Samples were collected from mesocosm experiments conducted from 13 May through 30 May 2017 at the National Mesocosm Facility located at the Espeland Marine Biological Station at the Raune-fjord (60o22.1'N, 5o28.1'E), University of Bergen, Norway. Six polyethylene enclosures measuring 2 m diameter, 8 m deep, hereafter referred to as mesocosms, were moored to a raft approx. 200 m from shore. During assembly, approximately 20,000 L of unfiltered fjord seawater was enclosed in each mesocosm and monitored for 17d. Three of the six mesocosms were amended on two consecutive days with pulses of inorganic nitrogen and phosphorus in Redfield ratio proportions in order to induce a phytoplankton bloom (total additions: 4 mM nitrate, 0.25 mM phosphate; hereafter referred to as replete mesocosms). Mesocosms were bubbled with ambient air for two days after nutrient additions to facilitate mixing. Mesocosms were monitored daily and

average temperature fluctuations at 1 m depth ranged from 10 to 11 oC.

2-heptyl-4-quinolone (HHQ) addition experiments:

Water obtained from replete mesocosms was spiked with 2-heptyl-4-quinolone (HHQ) or solvent vehicle controls every two days over a 15 d period to examine the effects of HHQ on microbial abundance and community structure over the course of an induced phytoplankton bloom. A 5 L Niskin bottle was used to collect mesocosm water from 1 m depth before passage through a 200 mm mesh filter to remove larger zooplankton. Equal volumes were collected from the triplicate replete mesocosms, pooled into 20 L carboys, and transported immediately to a 10 oC cold room for further processing. The water collected was dispersed among nine, 4.7 L polycarbonate bottles that had been acid-washed and rinsed in 18.2 mW water (Millipore Milli-Q). Triplicate bottles representing time zero controls were immediately processed for determination of chlorophyll a concentration, cell enumeration, and nucleic acid acquisition (details below). The remaining bottles were amended in triplicate with either 410 nM (100 ng mL-1) HHQ dissolved in dimethyl sulfoxide (DMSO) or an equal concentration (0.1% v:v) of DMSO to serve as a solvent vehicle control. These six bottles were mixed well before incubation for 24 hr in a land-based mesocosm containing flow-through surface seawater matching in situ temperatures. Window screen shading was used to replicate light levels (7,500 lux) corresponding to a depth of 1 m in the fjord-based mesocosms. The total time between subsampling the mesocosms and incubating the bottles was kept under 1 hr. After 24 hr, the bottles were processed in a 10 oC cold room for determination of chlorophyll a concentration, cell enumeration, and for some experiments, nucleic acid acquisition as described below.

Biomass collection and DNA isolation:

Triplicate microbial biomass samples from the HHQ addition experiments were taken at T0 directly from the pooled mesocosm sample and at T24 after 24 hr exposure to either 410 nM HHQ or a DMSO (0.1 % v:v) solvent vehicle control. In a 10 oC cold room, microbial biomass was harvested by passing between 0.8 and 2 L of sample through a 1 µm polycarbonate filter followed by a 0.2 µm polycarbonate filter via serial filtration. The microbial communities collected on these filters are referred to throughout as particle-associated (1 - 200 µm fraction) and free-living (0.2 - 1 µm fraction) communities. A peristaltic pump system fitted with silicon tubing and filter holders was flushed with 18.2 mW water (Millipore Milli-Q) between samples to prevent sample carry-over. Each sample was filtered in less than 30 min, and immediately after filtration, filters were placed in cryovials, flash frozen in liquid nitrogen, and stored at -80 oC until DNA isolation.

DNA was isolated from 1 µm and 0.2 µm polycarbonate filters using an established protocol (Urakawa et al, 2010) with recent modifications (Biller et al, 2018), including steps to remove RNA contamination. Briefly, polycarbonate filters were thawed on ice and placed in lysing matrix E tubes containing 400 µl of phenol:chloroform:isoamyl alcohol (25:24:1, pH 8.0) and 400 µl of 2X TENS Buffer (100 mM Tris-hydrochloric acid at pH 8.0, 40 mM Ethylenediaminetetraacetic acid, 200 mM Sodium chloride, 2% Sodium dodecyl sulfate), agitated for 10 min using a horizontal vortex adapter, and centrifuged at 14,000 rpm for 6 min. The aqueous phase was carefully transferred to Phase Lock Gel (PLG) tubes (Quanta Bio) containing 375 µl chloroform, mixed via gentle inversion, and centrifuged at 14,000 rpm for 6 min. The supernatant was transferred to a sterile microcentrifuge tube and incubated with 0.5 µl of RNase A (100 mg/ml; Qiagen) at 37 oC for 30 min after mixing by gentle inversion. After RNase treatment, samples were transferred to a new PLG tube containing 300 µl of 7.5 M ammonium acetate and mixed by gentle inversion before the addition of 700 µl of chloroform and additional mixing by inversion. These tubes were centrifuged at 14,000 rpm for 6 min and the supernatant was transferred to a sterile microcentrifuge tube in which DNA was recovered by alcohol precipitation using 360 µl of ice-cold isopropanol containing 2 µl of linear acrylamide (5 mg mL-1; AMRESCO). Samples were mixed thoroughly by repeated inversions before incubating on ice for 1 hr. DNA pellets were formed by centrifugation (14,800 rpm for 15 min at 4 oC), at which point the isopropanol was removed and the DNA pellet was washed with 500 µl of ice-cold 75% ethanol. DNA pellets were again formed (14,800 rpm for 8 min at 4 oC) before removing the ethanol by decanting and drying the pellets in a laminar flow hood for 2-5 min. Pellets were resuspended in 40 µl of nuclease free water and the total DNA yield was quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific) with yields ranging from 0.2 - 4.5 µg total DNA.

Amplicon library preparation and sequencing:

In order to comprehensively examine how microbial community composition was impacted by HHQ over the course of the bloom, T0 DNA samples from all experiments, and T24 DNA samples from experiments 1, 3, 5, and 7 were chosen to prepare 16S and 18S rRNA gene amplicon libraries for sequencing. Libraries were prepared and sequenced by the Georgia Genomics and Bioinformatics Core at the University of Georgia. Libraries targeting the V4-V5 region of the 16S rRNA gene were constructed using the following primers: 515F (5’-GTGYCAGCMGCCGCGGTAA-3’) and 926R (5’-CCGYCAATTYMTTTRAGTTT-3’) to obtain longer amplicons, reduce biases against archaea and the SAR11 clade, and obtain eukaryotic plastid sequences (Walters et al,

2015; Parada et al, 2016). Libraries targeting the 18S rRNA gene were constructed using the following primers: Euk1391F (5'-GTACACACCGCCCGTC-3') and EukBr (5'-TGATCCTTCTGCAGGTTCACCTAC-3') to target microbial eukaryotic lineages (Amaral-Zettler, et al, 2009; Caporaso, et al, 2012). PCR amplification was performed following the protocols and standards recommended by the Earth Microbiome Project for preparation of 16S and 18S amplicons for Illumina sequencing (Caporaso, et al, 2012; earthmicrobiome.org) and libraries were prepared using procedures outlined in the Illumina 16S metagenomic sequencing library preparation guide (Illumina, 2018), using an input of 25 ng of DNA. Amplicon libraries were multiplexed in two sets of 72 and sequenced using the Illumina MiSeq platform to produce 300+300 nt paired reads. After demultiplexing, three samples were found to contain anomalously low information (<300 reads each) and were removed from further analysis. Of the remaining samples, a median total of approx. 150K raw paired-end reads were obtained for each sample (range: approx. 14K – 1.2 million due to variations in library loading).

Data Processing Description

Processing of amplicon reads and figure generation were done using R (V3.5.1; https://www.R-project.org). Pre-processing of reads and inference of amplicon sequence variants (ASVs) was performed using the DADA2 package (V1.9.1), providing de novo identification of high resolution exact sample sequences (Callahan, et al, 2016, Callahan, et al, 2017). After removal of primer sequence and inspection of read quality profiles, reads were truncated as follows to remove low quality nucleotides while maintaining sufficient (> 20 nt) overlap between paired reads: 16S reads truncated at 245 nt (forward) and 195 nt (reverse); 18S reads truncated at 200 nt (forward) and 180 nt (reverse). Reads were filtered and phiX contamination removed using DADA2 standard filtering parameters. Error rates were determined using the first billion bases of each dataset prior to dereplication and inference of ASVs. Paired reads were then merged (ca. 99% of reads successfully merged) and reads with unexpected lengths (> 1% of reads) as well as chimeras (ca. 2% of reads) were identified and removed using the default DADA2 parameters.

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- split lat and lon into separate columns

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Data Files

File
accessions.csv(Comma Separated Values (.csv), 10.24 KB) MD5:d042b46eeef912a244a7e08e9b7761f2 Primary data file for dataset ID 753232

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Related Publications

Amaral-Zettler, L. A., McCliment, E. A., Ducklow, H. W., & Huse, S. M. (2009). A Method for Studying Protistan Diversity Using Massively Parallel Sequencing of V9 Hypervariable Regions of Small-Subunit Ribosomal RNA Genes. PLoS ONE, 4(7), e6372. doi:10.1371/journal.pone.0006372
Methods

Amaral-Zettler, L. A., McCliment, E. A., Ducklow, H. W., & Huse, S. M. (2009). Correction: A Method for Studying Protistan Diversity Using Massively Parallel Sequencing of V9 Hypervariable Regions of Small-Subunit Ribosomal RNA Genes. PLoS ONE, 4(12). doi:10.1371/annotation/50c43133-0df5-4b8b-8975-8cc37d4f2f26
Methods

Biller, S. J., Berube, P. M., Dooley, K., Williams, M., Satinsky, B. M., Hackl, T., … Chisholm, S. W. (2018). Marine microbial metagenomes sampled across space and time. Scientific Data, 5, 180176. doi:10.1038/sdata.2018.176
Methods

Callahan, B. (2017). Asv Tables Inferred By Dada2 From The Tara Oceans V9 Metabarcoding Dataset [Data set]. Zenodo. https://doi.org/10.5281/zenodo.581694
Methods

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581–583. doi:10.1038/nmeth.3869
Methods

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., … Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal, 6(8), 1621–1624. doi:10.1038/ismej.2012.8
Methods

Illumina. 16S Metagenomic Sequencing Library Preparation: Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System. (2013). [cited 2018 Oct 18]; Available from: https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf https://www.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf
Methods

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2015). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology, 18(5), 1403–1414. doi:10.1111/1462-2920.13023
Methods

Urakawa, H., Martens-Habbena, W., & Stahl, D. A. (2010). High Abundance of Ammonia-Oxidizing Archaea in Coastal Waters, Determined Using a Modified DNA Extraction Method. Applied and Environmental Microbiology, 76(7), 2129–2135. doi:10.1128/aem.02692-09 https://doi.org/10.1128/AEM.02692-09
Methods

Walters, W., Hyde, E. R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., … Knight, R. (2015). Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys. mSystems, 1(1). doi:10.1128/msystems.00009-15 https://doi.org/10.1128/mSystems.00009-15
Methods

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Parameters

Parameter	Description	Units
bioproject_accession	NCBI BioProject identifier	unitless
biosample_accession	NCBI BioSample identifier	unitless
biosample_link	URL for SRA BioSample Page at NCBI	unitless
sample_name	Sample name	unitless
organism	NCBI taxonomy name	unitless
tax_ID	NCBI taxonomy ID	unitless
collection_date	Date sample was collected formatted as dd-Mon-yyyy	unitless
depth	Nominal depth of sample collection	meters
lat	sample collection latitude; north is positive	decimal degrees
lon	sample collection longitude; east is positive	decimal degrees
experiment	Experiment number	unitless
time	Microcosm incubation time	hour
amplicon	Gene targeted during amplicon library construction	unitless
filter_size	Pore size of filter used during biomass collection	micrometers (um)
description	Brief description of the most relevant attributes of each BioSample	unitless

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Instruments

Dataset-specific Instrument Name	Illumina MiSeq
Generic Instrument Name	Automated DNA Sequencer
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	
Generic Instrument Name	Niskin bottle
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

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Project Information

Collaborative Research: Building a framework for the role of bacterial-derived chemical signals in mediating phytoplankton population dynamics (HHQSignals)

Coverage: Bergen, Norway

NSF Award Abstract:
Bacteria and phytoplankton play a central role in the modification and flow of materials and nutrients through the marine environment. While it has been established that interactions between these two domains are complex, the mechanisms that underpin these interactions remain largely unknown. There is increasing recognition, however, that dissolved chemical cues govern these microbial interactions. This project focuses on establishing a mechanistic framework for how bacterially derived signaling molecules influence interactions between phytoplankton and bacteria. The quorum-sensing (QS) molecule, 2-heptyl-4-quinolone (HHQ) will be used as a model compound for these investigations. Previously published work suggests that exposure to very low levels of HHQ results in phytoplankton mortality. Gaining a mechanistic understanding of these ecologically important interactions will help to inform mathematical models for the accurate prediction of the cycling of material through the marine microbial loop. This work initiates a new, hybrid workshop-internship undergraduate research program in chemical ecology, with a focus

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Interactions between phytoplankton and bacteria play a central role in mediating biogeochemical cycling and microbial trophic structure in the ocean. The intricate relationships between these two domains of life are mediated via excreted molecules that facilitate communication and determine competitive outcomes. Despite their predicted importance, identifying these released compounds has remained a challenge. The PIs recently identified a bacterial QS molecule, HHQ, produced by globally distributed marine gamma-proteobacteria, which induces phytoplankton mortality. The PIs therefore hypothesize that bacteria QS signals are critical drivers of phytoplankton population dynamics and, ultimately, biogeochemical fluxes. This project investigates the timing and magnitude of HHQ production, and the physiological and transcriptomic responses of susceptible phytoplankton species to HHQ exposure, and quantifies the influence of HHQ on natural algal and bacterial assemblages. The work connects laboratory and field-based experiments to understand the governance of chemical signaling on marine microbial interactions, and has the potential to yield broadly applicable insights into how microbial interactions influence biogeochemical fluxes in the marine environment.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1657808
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